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Viral Hepatitis B: Seroprevalence and Genetic Diversity in Blood Donors North of Congo

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Abstract

Introduction: Viral hepatitis B is the most formidable and confusing of all viral hepatitis, given its cirrhogenic and carcinogenic potential. The objective of the study is to characterize the molecular profile of hepatitis virus B in northern Congo. **Materials and Methods:** This was a descriptive and cross-sectional study that was carried out between January and September 2014, that is 9 months, in the establishments of the National Center for Blood Transfusion (CNTS) of the 4 departments of the north of the Congo. Epidemiological, serological and molecular variables (HBsAg, HBV DNA, genotypes and subtypes) were studied. The HBs antigen was searched by rapid test and confirmed by ELISA. For all positive donors, conventional and specific gene extraction and amplification techniques were performed for the identification of genotypes and subtypes from the serum. **Results:** A total of 892 donors were included. The average age was 35.36 ± 12.36 years with extremes ranging from 18 to 65 years old. The sex ratio (M/F) was 3.3. The prevalence of HBV was 8.6%. The viral DNA of HBV was amplified in 83.1%. Four genotypes were found: E (40.6%), A (3.1%), B (3.1%) and C (1.6%). Two cases of co-infection were identified: E/A (15.6%) and B/C (1.6%). Subgenotypes A1, B2 and C1 were highlighted. **Conclusion:** The prevalence of HBV is high, HBV infection remains a major public health problem for blood donors in Congo. Genotypes E, A, B, C as well as subtypes A1, B2, C1 have been identified in northern Congo.

Keywords

HBV, Molecular Biodiversity, Subgenotypes, North Congo

1. Introduction

Infection with the hepatitis B virus is a major health problem in the world. It is estimated that about 400 million chronic carriers of hepatitis B virus (HBV) and more than one million deaths annually [1] [2] worldwide. In Africa, it is estimated that about 100 million people are infected with HBV [1]. In Congo, the prevalence of HBV carriage among blood donors is 7.8% with unequal distribution throughout the country [2]. The departments of southern Congo (Bouenza, Pointe-Noire, and Niari) are considered hyper-endemic areas for HBV. The identification of HBV reduces the risk of transmission and improves blood transfusion safety. The genetic diversity of HBV makes it necessary to identify HBV genotypes because HBV genotype research in recent years has shown significant associations between HBV genotypes and the severity of HBV, clinical outcomes, and response to antiviral treatments [3]. Several genotypes have been identified in the world, they are classified from A to J and have a distinct geographical distribution. In Congo, genotypes A and E are the most common [2] [4]. But, these data are incomplete and do not concern all departments. The aim of the study is to characterize the molecular profile of HBV in blood donors in northern Congo.

2. Materials and Methods

It was a descriptive and cross-sectional study that ran from January 1st 2014 to September 30th 2014, is a period of 9 months. The study was carried out in Congo in the establishments of the Interdepartmental Center of Blood Transfusion (CIDTS) of the northern zone of the country located in the following departments: the Plateaux (Gamboma), the Central Cuvette (Owando, Boundji, Oyo), the West Bowl (Ewo) and the Sangha for sample collection. The serological and molecular analyzes were carried out in Morocco in the Laboratory of Virology and Microbiology Quality/Ecotoxicology and Biodiversity (LVMQ/ETB) of the Faculty of Science and Technology (FST) of Mohammedia—Casablanca, Hassan II University. The transport was carried out as follows; first by car, in a cooler containing cold accumulators of the various post blood transfusion to the CIDTS of Owando, then Owando to national blood transfusion center (CNTS) of Brazza city on a distance of 510 km for 7 hours. All these samples were analyzed in Brazzaville. Then we transported the samples from Brazzaville to Mohammedia (Morocco) by air in the hold, in dry ice after CO_2 bombardment, for 8 hours and stored at −20˚C until analysis. The choice of health structures that served as a survey frame was random. The study population consisted of occasional, regular and family blood donors in the different study centers. Included were all donors between the ages of 18 and 65 who consented to the study and were considered fit to donate blood. Non-consenting donors and all those with inoperable sera (denaturation) either by default of storage or by default of transport of sera were excluded from the study. Sociodemographic, biological (HBsAg, HBV DNA, HBV genotype) and

risk factors for HBV transmission were studied. Serological analysis was performed from the serum stored at −20˚C. The fourth-generation ELISA technique allowed the detection of AgHbs using the Monolisa HBs Ag ULTRA brand BIORAD reagent. All HBsAg positive samples were molecularly tested using the QIAGEN QIamp DNA blood kit. DNA extraction was carried out on the HBV S surface gene from the HBPol primer pairs of the PreS1/PreS2/HBsAg, HBPr1/HBPr135 and HBPr2/HBPr3 domains. All samples positive for nested PCR were genotyped using the type-specific PCR method (TS-PCR) as described by Naito *et al*. [5]. Data development and processing were done with CSPRO 5.1, Excel, 2013 and SPSS 21. The proportions were compared with an appropriate Chi-square statistical test (X2) with a significance threshold $p < 0.05$.

3. Results

At the end of this survey, 892 blood donors were collected and distributed as follows: 320 (35.9%) to Owando; 173 (19.4%) in Gamboma; 141 (15.8%) in Ouesso; 133 (14.3%) at Ewo; 92 (10.3%) at Oyo; 33 (3.7%) at Boundji.

Family donors accounted for 67.9% (606 donors) of the study population. There were 197 (22.1%) regular donors and 89 (10%) volunteer donors. The average age of our study population was 35.36 ± 12.36 years with extremes ranging from 18 to 65 years old. Male donors accounted for 61.1% (545/892), while females accounted for 38.9% (347/892). The sex ratio was 1.57 or 157 men per 100 women (Table 1).

Unsafe sex, circumcision, and multiple sexual partners were the most common risk factors for transmission (Table 2). Of the 892 donors screened, 77 were positive for HBsAg, which is 8.6%. There were 61 (79.2%) family donors, 11 (14.3%) regular donors and 5 (6.5%) volunteer donors. Parmi les 77 échantillons positifs à l'AgHBs et soumis à l'étude moléculaire, 64 se sont révélés positif à l'ADN viral du VHB après PCR nichée. The overall molecular prevalence of HBV infection was 83.1%. Genotype A and co-infection E/A prevail (Table 3); the subgenotypes A1, B2 and C1 have been identified in identical ways.

Table 1. Distribution of patients by age group by gender.

Sex Age	M		F		Total	
	Effective	%	Effective	%	Effective	%
<25	138	15.5	87	9.8	225	25.2
25 - 34	140	15.7	90	10.1	230	25.8
35 - 44	131	14.7	69	7.7	200	22.4
45 - 54	109	12.2	79	8.9	188	21.1
≥55	27	3.0	22	2.5	49	5.5
Total	**545**	**61.1**	**347**	**38.9**	**892**	**100**

Khi2 = 3.152; P = 0.533 > 0.05.

Table 2. Risk factors of the study population.

Factor of risk	Effective	Percentage
Sexual risk ratio (unprotected)	211	23.7
Circumcision	280	20.2
Multiple sex partners	115	12.9
Scarification	99	11.9
Dental care	50	5.6
Tatoo	47	5.3
Surgical intervention	35	3.9
Piercing	25	2.8
Accidental injury with sampling needle	18	2.0
Transfusion	14	1.6
Partner Drug addict	7	0.8
Invasive exposure (endoscopy with or without biopsy)	5	0.6
IV drug use	3	0.3
Mother infected with hepatitis B	1	0.1

Table 3. HBV genotyping results by specific PCR.

AgHBs Positive Genotypes	Effective	Percentage
E	26	40.6
E/A	10	15.6
A	2	3.1
B	2	3.1
C	1	1.6
B/C	1	1.6
Not determined	22	34.4
Total	64	100

4. Discussion

The collection of samples in our study was done in a fixed cabin, that is to say in place of each blood establishment. This method makes it possible to avoid staining and confusion of the samples. The high prevalence of HBsAg in our series confirms Congo's place among the highly endemic countries of hepatitis B as reported by Atipo Ibara and al [2]. This prevalence can be explained by the absence of a policy to reduce the transmission of hepatitis viruses; Congo does not have a program to fight viral hepatitis [6]. The establishment of a viral hepatitis control program would fill the lack of information on the viral hepatitis B virus and the lack of popularization of the means of prevention against this virus. Similarly, immunization of HBV seronegative donors would be a better weapon against this infection. The prevalence of HBsAg is variously appreciated in the literature. It seems higher in the work already done in the Congo and in

our study but weaker in the work done by some African authors [2] [7] [8]. Depending on the type of donation, our study showed a significantly higher prevalence in family or replacement donors. This difference in prevalence could be explained by the loyalty efforts of regular and occasional donors. This supports the hypothesis that regular donors are at low risk of transmitting diseases through blood transfusion to the extent that they receive information about the importance of blood safety and are screened for each blood donation. However, despite CNTS's efforts to retain regular donors, our study reveals that 14.3% of regular donors carry HBsAg. This result could be explained by the serological window of HBV. This window, which is immunologically silent, separates the date of the contamination from the one in which the HBV markers appear, which justifies the negativity of the tests at this period, hence the interest of the PCR. Indeed, for Kone *et al*., the safety of blood transfusion would be ensured by strategies that should be directed towards the abandonment of family blood donation such as the promotion of volunteer donation, loyalty and the organization of donors in clubs [9]. Naila *et al*. claim that in Pakistan the risk of HBV transmission is higher among replacement donors (family) because this category is in fact associated with poor people, who are secretly paid by the family and who conceal information during the selection interview [10]. We found a significant difference between the carriage of HBsAg and the risk factors for transmission found in the study. Several authors make the same observation [11] [12]. Molecular biology techniques, long reserved for the field of research in sub-Saharan Africa, contribute qualitatively to the improvement of screening in blood transfusion. DNA is a marker of viral replication, those with a molecular signature are a reservoir of the virus and have a higher risk of developing chronic hepatitis and/or cirrhosis and/or hepatocellular carcinoma [2]. We found the E, A, B, C genotypes with a predominance of the E genotype. However, we noted the associations between the E/A and B/C genotypes. Our results are consistent with those found by Atipo Ibara *et al*. in a study conducted in southern Congo [2]. These results are in agreement with the literature, which states that genotype A is ubiquitous, predominating in northwestern Europe and central Africa [13]. The same is true for genotype E, which is more common in Central Africa and West Africa [14] [15]. The particularity of this study is the discovery of genotypes B and C that do not exist in southern Congo. However, genotypes B and C are mainly found in Asia and the Pacific Islands [16]. Their presence in northern Congo could be linked to the mixing of populations. Thus genotypes B and C would be import strains. The other particularity in the study is the identification of subtypes of HBV, in particular the subgenotypes A1, B2 and C1. However, given the very small number of sequences analyzed, we can not accurately define the sub-genotypes circulating in this area of the country. However, this preliminary result provides an overview of the epidemiological profile of genotypes and HBV genotypes in blood donors in our study population and can serve as a guideline in the development of transfusion safety

strategies in Congo.

5. Conclusion

The present study reveals that HBV is common among blood donors in the departments of Plateaux, Central Basin, West Cuvette and Sangha in northern Congo. These results are consistent with the previous study in the south of the country, which places Congo in the zone of high HBV endemic countries. We identified the genotypes A, B, C, E as well as the subgenotypes A1, B1, C1. The high frequency of HBV as well as its genotypic variability justify the establishment of a genomic denomination of blood donations in order to improve transfusion safety.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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